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The Role of Medial Temporal Lobe Structures in Implicit Learning: An Event-Related fMRI Study

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Summary

The medial temporal lobe (MTL) has been associated with declarative learning of flexible relational rules and the basal ganglia with implicit learning of stimulus-response mappings. It remains an open question of whether MTL or basal ganglia are involved when learning flexible relational contingencies without awareness. We studied learning of an explicit stimulus-response association with fMRI. Embedded in this explicit task was a hidden structure that was learnt implicitly. Implicit learning of the sequential regularities of the "hidden rule" activated the ventral perirhinal cortex, within the MTL, whereas learning the fixed stimulus-response associations activated the basal ganglia, indicating that the function of the MTL and the basal ganglia depends on the learned material and not necessarily on the participants' awareness.

Introduction

A fundamental function of learning is to allow organisms to detect and use regular structures inherent in the environment. Motor skills as well as cognitive skills like piano playing, speaking, and mental arithmetic require the online processing of structured sequences. The serial order of stimuli is determined by corresponding rules as well as by the actions of the organism experiencing the stimuli. Therefore, the sequential order of stimuli in the environment is almost never random. Rather, they follow some form of structure. The detection of such regularities allows the prediction of upcoming events and therefore allows for adaptive behavior. Several experiments have demonstrated that this type of learning can occur incidentally, i.e., in the absence of the capability to consciously report what was learned, and therefore has been termed implicit (for reviews see Reber, 1993; Seger, 1994) .

Prior research into implicit learning has often used paradigms that included either a structured sequence of stimulus presentations or motor responses. One task that has been used in examining implicit learning of sequential structures is the serial reaction-time task (SRTT; Nissen and Bullemer, 1987). In the SRTT a constant sequence of required motor responses yields a gradual reduction of reaction time (RT). Several authors hypothesized that implicitly learning a motor sequence in the SRTT may foster activation in motor-related cortical areas such as primary motor cortex (M1), supplementary motor area (SMA), and basal ganglia (Grafton et al., 1995; Hazeltine et al., 1997).

In a recent memory study, it was demonstrated that the motor system is also involved in learning probabilistic stimulus-response matching (Poldrack et al., 2001). Two versions of a category learning task ("weather prediction") with probabilistic stimulus-outcome relations were directly compared to disentangle brain areas involved in declarative and nondeclarative memory. For a given stimulus, subjects had to indicate the appropriate outcome by a button press. In one version, feedback (FB) was given after the button press, and the probabilistic cue-outcome relations emphasized nondeclarative memory processes. In the other version, subjects learned the stimuli and categories in a paired associates (PA) manner, where both stimuli were presented simultaneously. In contrast to the FB task, the PA condition is thought to engage more declarative strategies. The medial temporal lobe (MTL) was activated under the PA version of the task, whereas the basal ganglia were involved in the FB version. The authors concluded that MTL and basal ganglia acquire different types of information during learning: the MTL acquires flexible, relational knowledge, whereas the basal ganglia acquire inflexible, i.e., constant, stimulus-response associations.

An important question emerging from this study is which area is involved in implicit learning in the absence of fixed stimulus-response associations but in the presence of an underlying unknown abstract rule. In accord with the results from this recent memory study (Poldrack et al., 2001), one would assume that relational learning without fixed stimulus-response mappings is a function of the MTL and that learning such a hidden feature would activate the MTL. However, if this relational learning without fixed stimulus-response mappings is implicit, one would expect that the MTL is not involved, because many authors have argued that the function of the MTL is directly linked to conscious recollection of memories (Poldrack et al., 2001; Eldridge et al., 2000; Clark and Squire, 1998; Knowlton et al., 1996; Squire and Zola-Morgan, 1991), and therefore MTL should not be engaged in implicit learning. This interpretation was questioned by a study that demonstrated that amnesic patients with lesions of the MTL were impaired in learning contextual information although the memory was not accessible to conscious awareness (Chun and Phelps, 1999). However, a recent study failed to replicate this finding (Manns and Squire, 2001). Therefore, it remains unclear whether MTL structures are involved in implicit learning tasks.

In contrast to the MTL, the basal ganglia are engaged in a variety of implicit learning tasks (Poldrack et al., 2001; Grafton et al., 1995; Honda et al., 1998), but their function may be based on the extraction of stimulus-response associations.

Taken together, previous studies do not allow us to

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fully disentangle implicit from explicit learning independent of fixed stimulus response mappings as compared to abstract relational learning.

Behavioral experiments demonstrated that implicit learning of abstract sequences resulted in item-general knowledge that can be transferred to novel tasks (Woltz et al., 1996, 2000; Doane et al., 1999). Existing models of MTL and basal ganglia function do not allow unequivocal predictions for specific functions in implicit abstract rule detection. With respect to MTL and basal ganglia functions, two alternative hypotheses can be tested. First, in the absence of awareness about the content of learning (i.e., implicit learning), the basal ganglia might even be involved in the extraction of abstract relations. This would emphasize the role of basal ganglia in the nondeclarative memory system but would imply that the function is not solely based on the extraction of fixed stimulus-response relations. Second, the MTL might be relevant for the detection of abstract relations, even in the absence of explicit knowledge. This would imply that the acquisition of relational knowledge in the MTL does not necessary depend on conscious access to that memory.

To examine the extraction of sequential regularities independent from fixed perceptual or motor sequences, we used the number reduction task (NRT), which originates from Thurstone and Thurstone (1941; see also Woltz et al., 1996, 2000). The version of the NRT used in the current experiment differs in several aspects from the original task and was developed by Frensch and Haider (2002). Participants receive a string of eight digits on a computer screen. All strings are composed of the different digits "1," "4," and "9." No other digit could occur in the strings. Participants are instructed to process the strings pairwise from the left to the right (see Figure 1) by following two rules. The first rule, the "same rule," states that the result of two identical digits is the digit itself (e.g., "4 4" results in "4"). The second rule, the "different rule," states that the result of two nonidentical digits is the remaining third digit (e.g., "4 1" results in "9").

Learning to correctly use these two rules (i.e., the same rule and the different rule) resembles an explicit learning task requiring learning fixed stimulus-response mappings. In addition to these overt rules, an abstract "hidden structure" was implemented in the NRT. This implemented hidden structure allows us to contrast learning of fixed stimulus-response associations (i.e., learning to use the same-different rules) with implicitly learning an abstract hidden structure. The hidden structure is abstract, because irrespective of the concrete stimulus-response associations in a given trial, the response pattern in all trials follows the same underlying principle: in our case, the responses for the last three input positions were always the mirror image of the responses for the previous three input positions (in Figure 1, "X 9 1 4 4 1 9"). That is, the last three responses were always determined by the responses for input positions 2, 3, and 4. It is important to note that this regularity is confined to the responses, but not present in the displayed string. Thus, the embedded hidden structure is abstract, because it refers to a relation between input positions and not to a fixed response sequence as used in the SRTT (Nissen and Bullemer, 1987). In addition, the abstract hidden structure also implies that the final result of a trial (i.e., the last required input) is already determined by the second input. It is important to note that this pattern does not result in any overt perceptual or motor sequence that is repeated over trials. The regularity is not communicated to the participants, and thus the learning situation is incidental. To assess the development of explicit knowledge, we used two measures: a postexperimental questionnaire on the one hand, and an online behavioral measure on the other hand. The use of the latter is important because of the low sensitivity of verbal reports after learning (Shanks and St. John, 1994). Participants who gain explicit knowledge about the hidden structure during the experiment can reduce the number of inputs, because they know the final result of the whole string after the second input. This possibility was outlined to the participants in the instruction as an option to speed up trial processing, whenever they know the final result before entering all previous results. This strategy shift (reduction of responses) can be easily detected, and therefore the task allows controlling for explicit memory generation within the learning period (see Frensch and Haider, 2002).

Thus, this task allows the separation of two parallel learning processes. First, there is the learning of the same-different structure, which should be reflected by a general improvement regardless of input position, because it is based on fixed stimulus-response relationship. The second process, implicit learning, is assumed to result in an additional improvement for the last inputs due to the determination from the hidden rule and is based on implicit relational knowledge without distinct stimulus-response mappings. We tested both effects for the MTL and basal ganglia to disentangle functional specialization regarding implicit rule learning and skill acquisition based on fixed stimulus-response mappings. Based on previous studies on sequential learning, we also examined the superior parietal cortex (Hazeltine et al., 1997; Eliassen et al., 2001; Grafton et al., 1995) and the cerebellum (Eliassen et al., 2001).

If participants become more sensitive for the hidden structure inherent in the task, then they should show increasingly faster responses for the determined response positions 5, 6, and 7 (the repeated responses; Figure 1) across sessions. Further evidence for implicit learning of the abstract hidden structure would be if a violation of the hidden rule would lead to behavioral consequences. We tested this hypothesis in the second part of the experiment. After five sessions, we presented a modified sixth session that included strings violating the abstract hidden structure (but not the same-different structure) at the last input position for half of the trials (see Figure 1 for details). An increase in RT for the violated position would be indicative of implicit learning of the hidden structure. Besides increases in RT, we hypothesized that this violation will activate the prefrontal cortex (PFC). This hypothesis is based on functional neuroimaging studies that demonstrated that PFC is activated if learned expectancies are violated (Nobre et al., 1999; Fletcher et al., 2001). In a study on the execution or suppression of hand movements, it was shown that the ventrolateral-prefrontal cortex is activated if a prepared response to a certain stimulus had to be inhibited (Krams et al., 1998). To test the hypothesis that the PFC is engaged also in the response inhibition due to

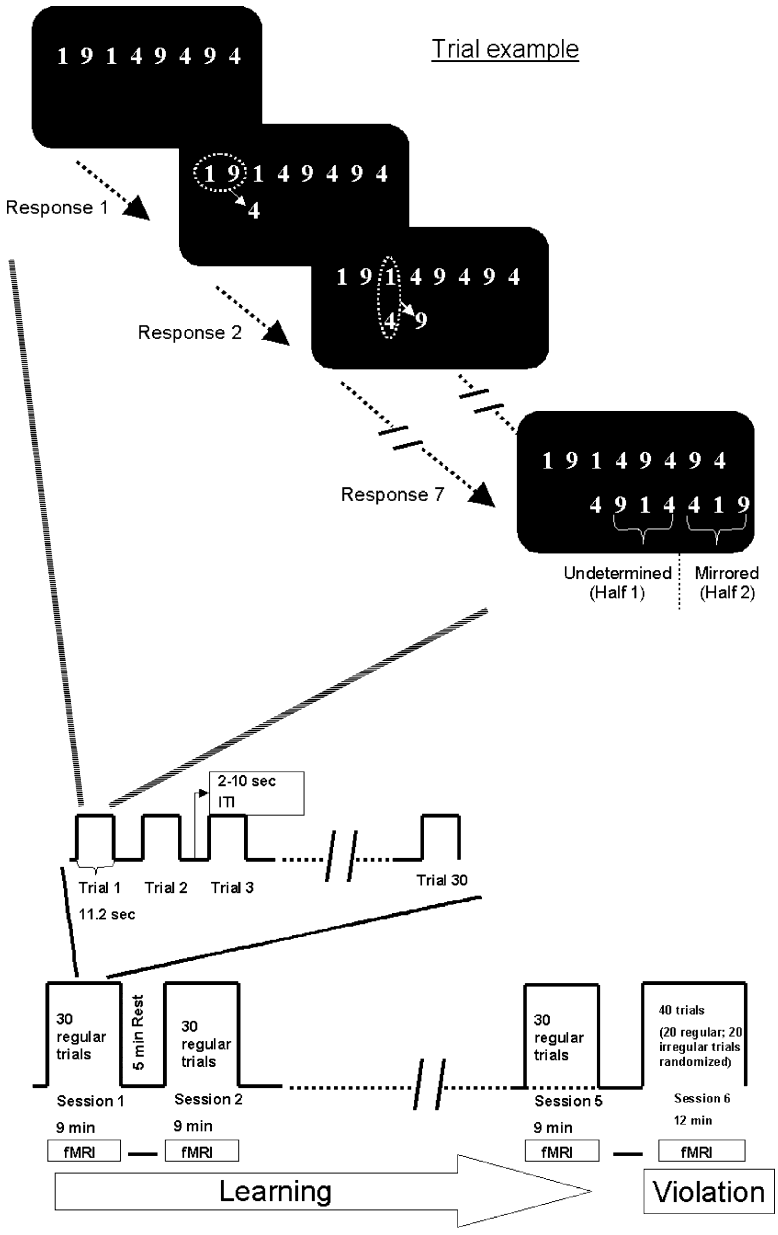

Figure 1. Task Example and Study Design

Example of a regular NRT trial with selected responses (top) and the design of the study (bottom). The abstract hidden rule ensures that the responses 5–7 (HALF 2) were the mirrored responses from positions 2–4 (HALF 1).

unpredictable events when learned memory is implicit, we compared responses that violated the hidden rule with those that follow the abstract hidden rule only within the last session.

Results

Behavioral Results

One out of ten participants demonstrated explicit knowledge about the hidden rule. This participant computed ten correct trials with only two inputs at the end of the second session and was able to explain the hidden rule. Based on these behavioral results, this participant was excluded from further fMRI analyses. None of the remaining nine participants processed a correct trial with less than seven inputs or reported any regularity in the questionnaire. In the postexperimental questionnaire two out of nine participants stated that they had the "feeling" that a structure was present in most trials, but were not able to verbalize any detail of this structure. Subjects were also asked to reproduce characteristic strings of responses after the study. However, apart from the subject excluded, no subject revealed the mirror symmetric configuration of responses 2, 3, 4 and 5, 6, 7.

Accuracy was calculated for each of the remaining nine participants as mean error rate per session. A one-way ANOVA indicated that participants reduced errors across sessions [$F(4,32) = 13.7$, $p < .001$]. Mean error rate decreased from session 1 (22%) to session 2 (7%) and remained constant in the third (5%), fourth (4%), and fifth sessions (7%). The violation of the hidden rule in Session 6 resulted in a statistical not reliable increase in error rate (11%, $t(8) = 1.3$, n.s.).

Mean RTs were calculated with respect to the appear-

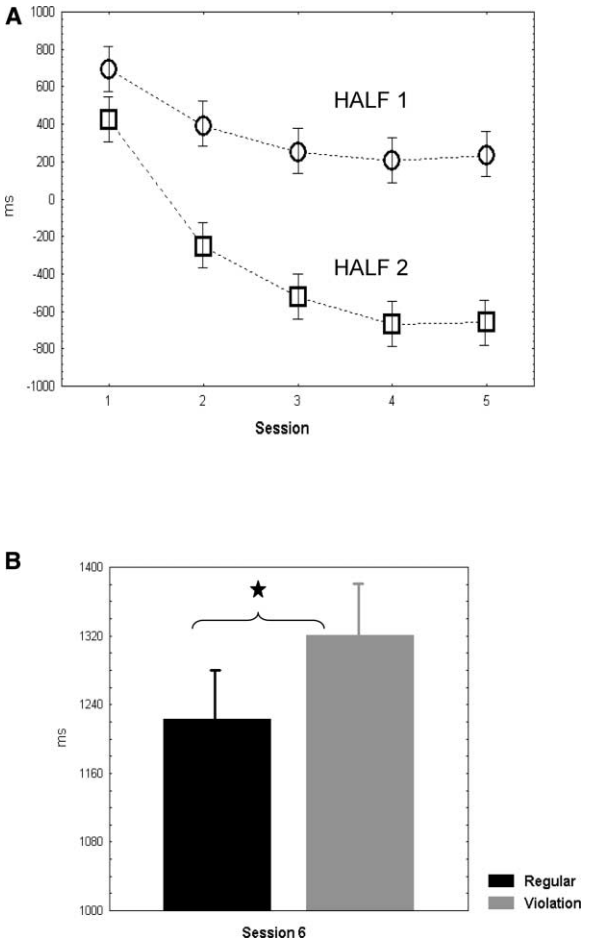

Figure 2. Reaction Times (RTs)

(A) Mean RTs for the undetermined inputs (HALF 1) and the inputs that were determined by the abstract hidden structure (HALF 2) across the five regular sessions. Negative values were due to the fact that RTs were measured with respect to the appearance of the previous result. As subjects get faster in responding they can respond before the previous result appeared on the screen, resulting in "negative" RTs. Skill acquisition is represented by the decreasing RTs for both HALF 1 and HALF 2; the faster RTs for the HALF 2 across session were due to the implicit learning of the abstract hidden rule.

(B) Time between input 6 and input 7 in Session 6 for regular trials and trials with a violation of the abstract hidden rule. The violation resulted in a reliable increase in the processing time of the last input (the asterisk indicates $p < 0.05$).

ance of the correct response for each single input and each session. A two-way ANOVA (factors position and session) revealed a general decrease of RTs across sessions [$F(4,36) = 14.1$, $p < .001$] and a difference between input position [$F(6,54) = 13.1$, $p < .001$]. The general decrease of RTs is in accordance with the assumed skill acquisition process for the same-different structure. Most importantly, we also found an interaction between session and input position [$F(24,216) = 8.1$, $p < .001$], indicating acquisition of the hidden rule. As shown in Figure 2A, the decreases of RTs across sessions for the determined last three input positions (HALF 2) were larger than for the undetermined input positions 2 through 4 (HALF 1). This indicates that the improvement in reaction time occurring from the first to the second half of a trial is more marked as the experiment progresses and subjects were exposed to the hidden rule.

Due to the hidden rule, the fifth response is always a direct repetition of the fourth response. It could be argued that the performance improvement for HALF 2 relies only on the detection of this simple relation, rather than on the complete hidden rule. To test this hypothesis, we compared RTs for inputs 2 and 3 (from HALF 1) and inputs 6 and 7 (from HALF 2) without the repeated responses. A three-way ANOVA (factors HALF, input position, and session) revealed a reliable interaction between session and HALF [$F(4,32) = 9.56$, $p < .05$], indicating that the performance improvement for HALF 2 is in fact due to the hidden rule and not only to the repetition effect of the fifth response. Furthermore, we explicitly asked the participants whether they detected the repeated response at position five in the postexperimental questionnaire. None of the included subjects reported awareness of this regularity. This gives strong grounds for attributing the performance improvement specifically to the presence of the rule, rather than merely to a nonspecific practice effect.

To additionally assess implicit learning of the abstract hidden rule independent of input position, we compared mean RTs of responses for input position 7 in regular versus irregular trials (i.e., trials in which the hidden rule was violated) in the sixth session. A t test revealed that RTs for input position 7 were faster when responses followed the hidden rule than when they did not [$t(8) = 2.8$, $p < .05$; Figure 2B]. This effect demonstrated that the abstract hidden rule affected processing of the digit strings, because irregular trials never violated the same-different rule. Taken together, the session by input position interaction across the five regular sessions and the increase of RTs due to the violation in Session 6 both indicate that participants become increasingly sensitive for the underlying abstract hidden structure.

Functional Neuroimaging

Skill Acquisition Process for the Same-Different Rule

We modeled each learning trial by two box-car functions, the first covering responses for the undetermined inputs 2–4 (HALF 1) and the second covering responses 5–7 (HALF 2), which were determined by the hidden rule. The general improvement in applying the same-different rule was accompanied by a linear increase in BOLD signal across session for both HALF 1 and HALF 2 in the basal ganglia and the cerebellum (Figure 3). The effect was more pronounced in the right hemisphere but also observed in the left hemisphere (Table 1).

To visualize the interaction between time (i.e., session) and BOLD response (Figure 3), we fitted a linear function (regression) to the data from HALF 1 and HALF 2 separately. As expected from the SPM analysis in the basal ganglia and the cerebellum, a reliable increase of signal intensity for HALF 1 and HALF 2 was observed [basal ganglia ($x = 27$, $y = 0$, $z = 0$ mm) HALF 1, $F(1,35) = 19.6$, $p < .05$; HALF 2, $F(1,35) = 16.1$, $p < .05$; cerebellum ($x = 21$, $y = -66$, $z = -30$ mm) HALF 1, $F(1,35) = 12.2$, $p < .05$; HALF 2, $F(1,35) = 25.0$, $p < .05$].

To link the observed effects more directly to behavior, we performed an additional regression analysis and used performance as indexed by the individual mean

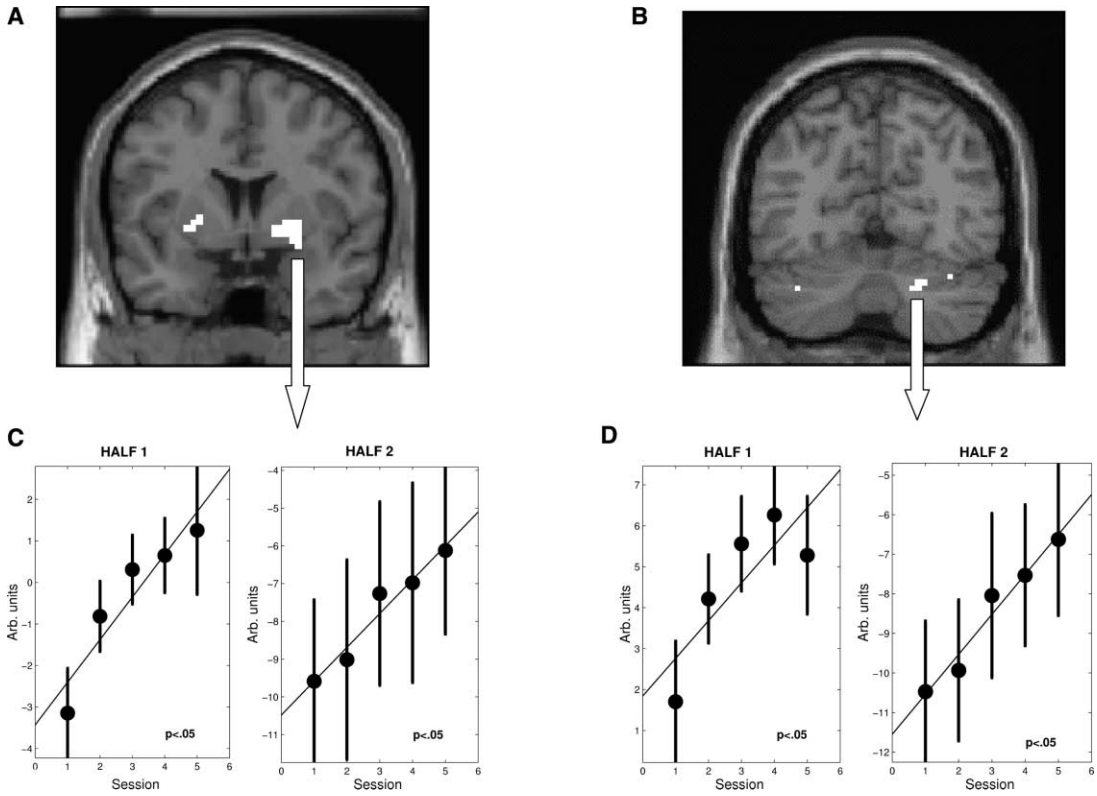

Figure 3. Skill Acquisition of the Same-Different Rules

(A and C) Effects of skill acquisition for the same-different rules in the basal ganglia (A) and mean regression coefficients (C) across the five regular sessions for HALF 1 (left side) and HALF 2 (right side) at [27 0 0 mm] (right basal ganglia). Note that regression coefficients have arbitrary units. The skill acquisition resulted in increasing BOLD signal for both HALF 1 and HALF 2 across sessions. The p values reflect the tested linear trend across sessions for HALF 1 and HALF 2.

(B and D) Corresponding effects in the cerebellum and mean regression coefficients at [21 −66 −30 mm].

RT for each session as a covariate rather then session number (i.e., time) as in the previous analysis. This analysis directly evaluates the relationship between performance and BOLD response. The sign of RTs was changed so that they reflect performance.

For the basal ganglia, the BOLD response was positively related to performance [HALF 1, $F(1,35) = 6.3$, $p < .05$; HALF 2, $F(1,35) = 4.3$, $p < .05$]. A similar result was obtained for the cerebellum [HALF 1, $F(1,35) = 7.5$, $p < .05$; HALF 2, $F(1,35) = 8.5$, $p < .05$].

Implicit Learning of the Hidden Rule

As demonstrated by the RT analyses, the interaction between input half (HALF 1 versus HALF 2) and session expresses the implicit learning of the abstract hidden rule. A linear interaction contrast was used to test for an equivalent effect across the five regular sessions.

Table 1. Coordinates (mm) and Magnitudes of Main Activations

	t	x	y	z
Implicit Learning of the Hidden Rule				
Right superior parietal	6.35[a]	12	−66	63
Left superior parietal	6.09[a]	−12	−66	57
Right MTL	4.84[a]	39	−18	−21
Left MTL	4.43[a]	−39	−15	−24
Skill Acquisition for Same-Different Structure				
Right basal ganglia	10.83[a]	27	0	0
Left basal ganglia	5.08[a]	−21	0	0
Right cerebellum	8.25[a]	21	−66	−30
Left cerebellum	7.42[a]	−30	−60	−30
Violation Effect in Session 6				
Right ventro lateral prefrontal	4.29[a]	54	39	0

Results from the random effects analysis with SVC (10 mm^3). Areas demonstrating an effect of implicit learning of the hidden structure, of skill acquisition for the same-different rules, and of the violation of the hidden rule in session 6 ([a]$p < 0.05$).

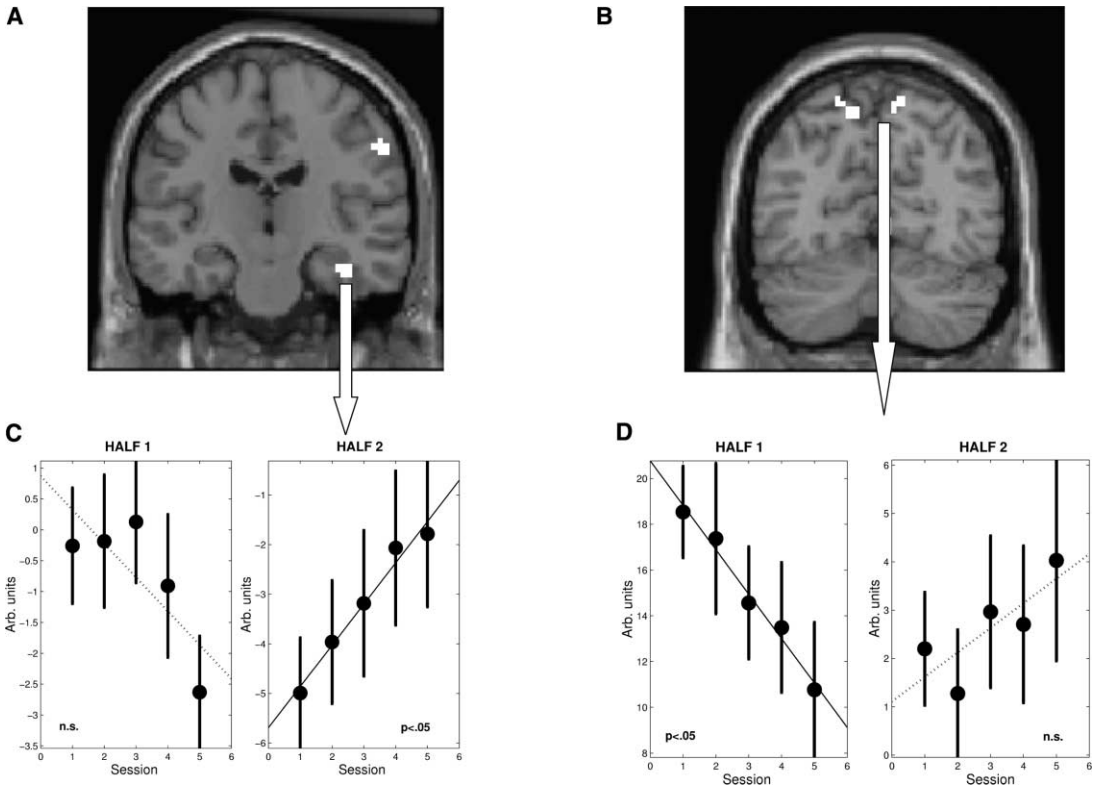

Figure 4. Implicit Learning of the Abstract Hidden Rule in the MTL and SPL

(A and C) Effects of implicit learning of the hidden structure in the right MTL (A; ventral perirhinal cortex) and mean regression coefficients (C) across the five regular sessions for HALF 1 and HALF 2 at [39 −18 −21 mm]. In contrast to the skill acquisition process, the implicit learning of the abstract hidden rule resulted in an increase across sessions of BOLD signal for HALF 2 but not for HALF 1. The p values reflect the significance of the linear regression analysis across sessions.

(B and D) Effects of implicit learning of the hidden structure in the superior parietal lobule (B; SPL) and mean regression coefficients (D) across the five regular sessions for HALF 1 and HALF 2 at [−12 −66 57].

Activation in the MTL (ventral perirhinal cortex) increased for the second half of inputs, but not for the first half of inputs across sessions (Figure 4A).

Additional areas with signal changes indicating a reliable interaction effect between input half and session were found in bilateral superior parietal cortex (SPL). In contrast to the MTL, BOLD responses only minimally increased for the determined inputs of HALF 2, but decreased for the undetermined inputs in HALF 1 (Figure 4B).

To visualize the interaction between time (i.e., session) and BOLD response in the MTL and SPL, we fitted a line to the data from HALF 1 and HALF 2 separately (Figure 4). This regression revealed a reliable increase in BOLD signal intensity in the MTL over time only for the determined responses from HALF 2 [($x = 39$, $y = -18$, $z = -21$ mm); $F(1,35) = 12.4$, $p < .05$], but not for the undetermined responses from HALF 1 [$F(1,35) = 2.6$, n.s.]. In SPL we found a significant decrease of BOLD responses over time for HALF 1 [($x = 12$, $y = -66$, $z = 63$ mm); $F(1,35) = 20.6$, $p < .05$] but no significant relationship either positive or negative for HALF 2 [$F(1,35) = 2.1$, n.s.].

To link the observed effects more directly to behavior, we performed an additional regression analysis and used performance as indexed by the mean RT for each session as a covariate rather then session number (i.e., time) as in the previous analysis.

In accord with the differential interaction (Figure 4A) in the MTL, a significant positive relationship with the individual performance was obtained for HALF 2 [$F(1,35) = 17.5$, $p < .05$] but not for HALF 1 [$F(1,35) = 1.5$, n.s.], indicating a selective involvement of MTL for the determined inputs (HALF 2).

For the SPL we found a negative relationship of the BOLD signal change and performance in HALF 1 [$F(1,35) = 8.6$, $p < .05$]. For HALF 2 a nonsignificant trend toward a positive relationship was observed [$F(1,35) = 1.3$, n.s.].

Effects of Violating the Hidden Rule

In session 6, we introduced two additional conditions: HALF 1 and HALF 2 of those trials that violated the hidden rule (50% of all trials in Session 6). We then compared HALF 2 of those trials that violated the hidden rule with HALF 2 of those trials that did not violate the rule only for the sixth session. This analysis revealed greater responses in the right ventrolateral prefrontal cortex (VLPFC) for responses that violated the hidden rule than those which followed the hidden rule.

Discussion

Our behavioral data provides evidence that the hidden rule was learned implicitly (i.e., in the absence of aware-

ness). In contrast to previous studies, the medial temporal lobe was engaged in learning these abstract relations, even though that knowledge was consciously not accessible, i.e., implicit. Furthermore, the behavioral data revealed a general improvement across sessions regardless of the hidden rule that reflected the acquisition of the same-different rule. This skill acquisition process is based on practicing fixed stimulus-response associations and increasingly activated the basal ganglia and cerebellum. This functional separation of the two learning processes indicates that the involvement of the MTL and the basal ganglia in learning depends on the content of memory but is not necessarily linked to participants' awareness.

The employed online measure, sensitive to the possible strategy shift, has the advantage that it can be used to detect explicit knowledge generation during learning. Based on this measure, we were able to exclude one participant who indeed acquired explicit knowledge about the hidden rule. All other participants showed no evidence for explicit awareness for the abstract hidden rule. Neither an early termination of trials (see Experimental Procedures) was observed, nor any knowledge about the abstract hidden rule was reported afterwards. This accords with extensive behavioral data on this task (Frensch and Haider, 2002). There was behavioral evidence for increasing sensitivity for the structural properties (i.e., hidden rule) of the task. Although no overt perceptual or motor sequence was repeated over trials, behavioral results indicated robust implicit learning. The practice of the same-different rule resulted in a reduction of RTs across sessions for all input positions. More importantly, implicit learning of the hidden structure resulted in RTs that reflected the determination of the last three inputs without awareness of the participants. The interaction between input half and session demonstrated that the last three determined inputs became faster than the previous three across sessions. The increasing sensitivity to the hidden rule demonstrated that participants implicitly learned the associative relation between inputs. In theory, it could be argued that the shorter RTs for the last inputs were simply due to the fact that they were the final responses in each trial. However, this alternative hypothesis is highly unlikely given the data from the sixth session. If the RT decrease found for HALF 2 only reflected the specific input position of responses, then regular and irregular responses for input position 7 should have been identical. Even in the irregular trials, the responses to input position 7 always followed the same-different rule and appeared at the same sequential position. Irregular trials differed, however, with respect to their concordance with the hidden rule. Nevertheless, RTs were reliably higher when the hidden rule was violated at the last input position. This is additional evidence for implicit knowledge of the abstract hidden rule.

The interaction between input half and session represents the process of implicit learning. On the basis of this interaction, we identified brain areas with comparable modulation of activity across sessions. With this contrast, the effects of practicing the given rules and habituation were excluded. Our data showed that implicit learning was accompanied by an increase in BOLD signal in the MTL and superior parietal cortex for the determined last inputs, but not for the first half of responses.

The observed effect in the MTL is in accord with previous data showing that the MTL acquires flexible, relational knowledge (Poldrack et al., 2001; Cohen et al., 1997). Support for this proposed function comes from the present study in which the MTL was engaged in the extraction of the abstract relations implemented by the hidden rule, as indicated by an increase in BOLD response as a function of time, and more importantly also as a function of performance improvement. For the nondetermined inputs from HALF 1, no modulation over time or related to performance was detected.

Many studies have linked hippocampal involvement to task awareness. In trace eyeblink conditioning, which is hippocampus dependent (McGlinchey-Berroth et al., 1997; Solomon et al., 1986) the strength of conditioning correlates with task awareness (Dusek and Eichenbaum, 1997; Clark and Squire, 1998; Eichenbaum, 1999; Manns et al., 2001). In the present study the involvement of the MTL did not depend on explicit access to the formed memory contents. This result is in accord with the finding that amnesic subjects with hippocampal and adjacent temporal cortex damage showed normal skill learning but were impaired on implicit contextual learning (Chun and Phelps, 1999). The patients were impaired in learning novel spatial configurations in a task where normal controls benefited from implemented context-target associations without explicit memory for associations. Therefore, it was concluded that the function of the medial temporal lobe is contextual—or more generally relational—encoding, independent of awareness. The same paradigm was subsequently used in a different study on amnesic patients (Manns and Squire, 2001). They divided the patients mainly into two groups: H^+, in which lesions were mainly restricted to the hippocampal region, and MTL^+, in which there was extensive damage to the MTL. Subjects in MTL^+ were impaired in the implicit memory task, whereas patients in H^+ performed normally. This finding is in accord with our data since the perirhinal cortex was damaged in the MTL^+ group, but not in the H^+ group.

The present result supports the view of functional differences between MTL structures. In our data, the MTL activation related to implicit learning of the hidden rule was located in the ventral perirhinal cortex but not in the hippocampal formation. However, it remains possible that an effect as detected by fMRI is ancillary and thus the ventral perirhinal cortex might not be essential for implicit learning.

Our data is further compatible with the hypothesis that explicit knowledge about the hidden rule may further enhance performance and MTL activation. In previous behavioral and ERP studies (Frensch and Haider, 2002; Rose et al., 2001), participants with explicit knowledge about the hidden rule in the NRT demonstrated reliably faster RTs than participants who only had implicit knowledge. This RT difference between participants who became aware of the task regularity and those who did not is due to the fact that the former are able to substantially speed up their responses by entering responses 5, 6, and 7 in quick succession. Nevertheless, the present data show reliable learning even in the absence of

awareness about the hidden rule and also show effects in the MTL paralleling this process.

The model of Nadel and Moscovitch (1998) states that reactivation and rehearsal of memories causes the formation of multiple memory traces within the hippocampal complex. It was argued that episodic memory is based on the integration of spatial and temporal information (Wallenstein et al., 1998; Eichenbaum, 2001). Many studies have shown that the integration of spatio-temporal information and the encoding of event sequences is the core function of the hippocampal complex relating its function to episodic memory (Eichenbaum, 1997, 2000).

Encoding of spatio-temporal information and event sequences are both relevant in extracting sequential regularities as implemented in the NRT task. In contrast, the need for an executive or conscious mechanism is not an obligatory consequence for the proposed MTL functions. Instead, it is possible that only under certain circumstances this detected relationship results in awareness and therefore creates the basis of episodic memory. Our results suggest that the perirhinal cortex is also engaged in the acquisition of constant relations that are accessed only in an implicit way.

Previous studies of implicit learning did not find MTL involvement in extracting sequential regularities in the SRTT. Main effects of learning in the SRTT were mainly found in the basal ganglia (Grafton et al., 1995; Hazeltine et al., 1997; Rauch et al., 1995). Interestingly, in our study, the basal ganglia did not exhibit activation related to the implicit learning of the hidden rule. However, effects of practice of the same-different rule increasingly activated the basal ganglia and the cerebellum. This finding speaks against the possibility that the hidden rule was learnt through motor representations. Our data are in accord with previous findings showing that the function of the basal ganglia depends on the acquisition and proceduralization of fixed stimulus-response mappings (Poldrack et al., 2001; Blazquez et al., 2002; Platt, 2002). It was demonstrated that this function also does not depend on declarative memory and that the basal ganglia are involved even in the extraction of probabilistic stimulus-response mappings (Poldrack et al., 2001). This is in accord with findings that demonstrated impaired performance of patients with Parkinson's disease in a probabilistic classification task (Knowlton et al., 1996). The authors concluded that the basal ganglia are part of a learning system that forms the basis of nondeclarative memory and that the MTL region is part of the declarative system. In contrast, the present results show that the practice of the explicitly given same-different rule involves the basal ganglia and the extraction of the sequential regularity of the hidden rule engages the MTL region although no declarative memory was generated.

Implicit learning was further accompanied by an effect in the superior parietal lobe (SPL). As demonstrated in Figure 4B, this effect was mainly due to a decrease of activity for the first half of inputs and a not-reliable increase of activation for the second half. Many functional brain imaging studies have revealed the SPL as an important region that is involved in visual attention (Kastner et al., 1999; Kastner and Ungerleider, 2000; Büchel et al., 1998). It is thus possible that attentional demands are greater for HALF 1 (undetermined inputs) as opposed to the determined HALF 2 and that this attentional load decreased over time in HALF 1 as the same-different rule becomes automatic.

Activation of the SPL has previously been linked to spatial processing. This could suggest that implicit learning of the hidden rule in our paradigm also has a spatial component. A possibility would be the mirrored appearance of input stimuli at positions 2, 3, 4 and 5, 6, 7. This hypothesis is in accord with recent data showing that implicit motor sequence learning (SRTT) at least in part depends on the sequence of response locations (i.e., spatial location) rather than the sequence of finger movements (Willingham et al., 2000).

The violation of the hidden rule in Session 6 activated a region located in the right ventrolateral prefrontal cortex (VLPFC). In the irregular trials, only one input from the second half did not follow the hidden rule, but was completely correct with regard to the given same-different rule. This small violation resulted in a greater BOLD signal in right VLPFC for the second half in the irregular trials than in the regular trials of Session 6. Previously, right VLPFC activation was found during tasks with response competition or inhibition of a planned motor response (Krams et al., 1998; Hazeltine et al., 2000; Konishi et al., 1999). However, in all these studies the subjects were fully aware of the conflict. Based on our data one could speculate that even a nondeclarative violation can lead to VLPFC activation. However, this claim needs further testing. Although expected on the basis of performance monitoring and error detection (MacDonald et al., 2000; Botvinick et al., 1999), no effects were found in the anterior cingulate cortex (ACC).

Conclusion

Our design allowed the dissociation of implicit learning about the hidden structure from the practice effect of the same-different rule. Implicit learning of the sequential regularities of the hidden rule engaged the MTL, whereas the practice of fixed stimulus-response associations involved basal ganglia and the cerebellum. These results indicate that the functional segregation of the MTL and the basal ganglia can depend on the type of material that is learned and not necessarily on the participants' awareness for stimulus contingencies. The present results suggest a possible role of MTL structures in the extraction of sequential relationships, even when the "knowledge" is only implicit.

Experimental Procedures

Subjects and Imaging

Ten healthy subjects (mean age 26 years, range 22–39 years, 5 females) participated in the study. All subjects were right-handed and had normal or corrected-to-normal vision. The study was approved by the local ethics committee and subjects gave written informed consent prior to the experiment.

Functional MRI was performed on a 1.5T system (Siemens Vision) with a gradient-echo EPI T_2* sensitive sequence in 32 contiguous axial slices (3 mm thickness with 1 mm gap, TR 2.6 s, TE 40 ms, flip angle 90°, field of view 210 × 210 mm^2, matrix 64 × 64). For display purposes, a high-resolution (1 × 1 × 1 mm voxel size) structural MRI was acquired for each participant using a standard 3D T_1-weighted FLASH sequence.

Stimuli were presented in white on a gray background controlled

by a PC that ensured synchronization with the MR-scanner using the software "Presentations" (http://www.neurobehavioralsystems.com). An LCD projector projected the stimuli on a screen positioned on top of the head coil which was viewed by the subjects through a mirror ($10^\circ \times 15^\circ$ field of view). Participants entered the responses by pressing buttons on a MR-compatible device.

Task

Participants saw a string of eight digits on the screen (Figure 1). Strings were always composed of three different digits ("1," "4," and "9"). Participants were asked to process the stimuli pairwise from the left to the right by applying the same-different rule. The same rule states that the result of two identical digits is the digit itself (i.e., "4 4" results in "4"). The different rule states that the result of two nonidentical digits is the remaining third digit (i.e., "1 4" results in "9"). First, the two leftmost digits of a given string are processed (in the example, the digits "1" and "9"). According to the different rule, stating that the result of two different digits is the remaining digit, the first pair provides "4" as the result. After 1.6 s the correct result was displayed below the third digit. The task of the participants was to respond before the result appeared, however. All remaining comparisons are now made between the preceding result and the next digit. Next, the result of the first comparison, "4," is compared with the next digit in the string, that is, the third digit in the sequence (the "1"). The result of this comparison is, according to the different rule, the digit "9." Next, this result (i.e., the digit "9") is compared to the next digit in the string, a "4." Comparing the digits "9" and "4" results, again according to the different rule, in a "1." On any given trial, participants generated and entered a total of seven responses. Only the excluded participant who generated explicit knowledge entered the final result after two responses in session two.

Overall, 54 eight-digit strings were constructed with a hidden structure in the response pattern of the form "x a b c c b a" ("x," "a," "b," and "c" representing the digits "1," "4," and "9"). Thus, the last three responses were always the mirrored repetition of the responses at positions 2–4 regardless of the exact digits. The material was constructed to avoid any other direct repetition of a response than the repetition of the response at position 5 that is due to the hidden rule. It is important to note that the regularity in the response pattern does not correspond with any overt structure in the presented stimulus string. For example, the digit strings "1 4 1 9 4 9 4 9" and "1 4 4 4 4 1 4 4" both result in a response string that followed the hidden rule (i.e., "9 4 1 9 9 1 4" and "9 1 9 1 1 9 1") but do not share any other structural characteristic. An important feature is that due to the hidden structure, in each trial the first half of inputs is not determined whereas the second half of inputs can be predicted by previous responses. The participants received no information about the principle underlying the construction of the strings.

To control for explicit memory generation, we included an opportunity to complete the trial without entering all inputs. The instruction stated that if a participant knew the final result (the last input of a trial) without processing all single inputs, the final result can be entered and the trial can be completed by pressing a fourth special button. This does not necessarily imply a hidden structure, as it is theoretically possible to apply the same-different rule successively for the whole string without entering intermediate results. However, given the time constraint of 1.6 s for each response, this is impossible, but leaves the option for participants with explicit knowledge to enter the final response after the second response directly.

After the experiment, all participants received a postexperimental questionnaire in order to further assess explicit knowledge about any regularities detected in the task. Participants had to rate the appearance of different regularities (perceptual, motor, or rule-like) on a four-item scale ("none," "sometimes," "mostly," "always"). If any regularity was observed, the participants were asked to write down a description of the observed regularity and try to reproduce characteristic input sequences. Finally, it was explicitly asked whether the participant realized the presence of the double response (the regular repetition from input 4 to input 5).

In the second part of the experiment (Session 6), we directly assessed the degree of implicit knowledge about the abstract hidden structure. For half of the trials in the violation session (40 trials total), the digit strings were manipulated only at the last position, resulting in an almost identical response pattern. Only the last response deviated from the pattern of the hidden rule. Thus, the last response was no longer determined by the response for input position 2 (e.g., if "4 9 1 4 4 1 9" was the original response pattern, the new pattern was "4 9 1 4 4 1 4"). Although this resulted in a violation of the hidden rule, the response was always in accord with the same-different rule. Reaction times and accuracy was assessed for all inputs. Comparisons were made between the regular and irregular trials of the sixth session.

Design

Each trial consisted of the processing of one eight-digit string that requires the calculation of seven responses. If explicit knowledge about the hidden rule was generated, the number of inputs can be reduced to compute the final result. The time limit for each input was 1.6 s, resulting in a trial duration of 11.2 s (7×1.6). The length of the intertrial interval (ITI) with a fixation cross on the screen was randomized between 2 and 10 s and served as a baseline condition. For each regular session, 30-digit strings were randomly chosen from the regular list (duration about 9 min per session). Each session was followed by a rest period of 5 min (cf. Figure 1). The sixth session was slightly longer and consisted of 20 trials from the regular list and 20-digit strings with a violation at the last position.

Image Processing and Statistical Analysis

Image processing and statistical analysis were carried out using SPM99 (http://www.fil.ion.ucl.ac.uk/spm). All volumes were realigned to the first volume, spatially normalized to a standard EPI template (SPM99), and smoothed using a 10 mm full-width at half-maximum (FWHM) isotropic Gaussian kernel. The structural volume was coregistered to the functional scans by normalizing it to a T_1-weighted template in the same space as the template used to normalize the functional data set.

Data analysis was performed by modeling the first half of responses that were not determined and the second half that were determined by the first inputs as box-car functions with variable duration convolved with a hemodynamic response functions (HRF). The duration was computed by the individual processing times for the first and the second half of inputs for each trial. An additional regressor was created for the error trials modeling the whole trial as a regressor with fixed duration (11 s). Regression coefficients for all regressors were estimated using least squares within SPM99 (Friston et al., 1995). A high-pass filter with a cut-off period of 120 s and a low-pass filter (Gaussian envelop FWHM of 4 s) were used.

For the group analysis, a second level analysis was performed, treating intersubject variability as a random effect. The threshold adopted was $p < 0.05$ (corrected for multiple comparisons). In regions with an a priori hypothesis, a small volume correction (SVC) was performed. For regions with an a priori hypothesis (MTL, SPL basal ganglia, cerebellum, and VLPFC), the correction was based on a search volume of 1000 mm^3. For the VLPFC we centered the volume of interest on the coordinate reported in a previous study (Krams et al., 1998) on response inhibition (58, 32, 8 mm)

To test hypotheses about regionally specific condition effects, the estimates were compared using linear contrasts for interaction effects of the first half and the second half of each trial. As described above, this interaction effect reflects the implicit learning of the hidden rule without the practice effect of the given same-different rule.

To assess the general improvement in applying the same-different rule (decreasing RTs for all inputs), a contrast modeling an increase for the first and second half of each trial was estimated.

It should be noted that categorical differences between HALF 1 and HALF 2 are confounded by experimentally dictated differences between those two conditions. For instance, HALF 1 always precedes HALF 2. Such differences between HALF 1 and HALF 2 were unavoidable in this paradigm, because the determined responses cannot precede the undetermined responses (i.e., HALF 2 has to follow HALF 1).

However, the introduction of a categorical difference between HALF 1 and HALF 2 was acceptable, since learning is reflected by the performance (or time) by condition interaction rather than by

the main effect of condition. The interaction is not confounded by the categorical difference. Consequently, we only interpreted the interaction of BOLD response with learning (slope) and contrasted the "HALF 1 by learning interaction" with the "HALF 2 by learning interaction," without interpreting the intercept of the regression curves, which is confounded by the categorical difference.

For the sixth session, the correct trials (out of 20) with the violation of the hidden rule were modeled separately with the first and the second half of inputs (resulting in five regressors for session 6: regular HALF 1 and HALF 2, irregular HALF 1 and HALF 2, and error trials). To test the effect of the violation of the hidden rule, the second half of inputs were compared between regular and irregular trials of the sixth session.

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